



# Exhibit A

ATTORNEY DOCKET NUMBER: 0342941-0065 (HU01275-96CON)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Ptashne et al.  
Serial No.: NOT YET ASSIGNED  
Filing Date: August 31, 2001  
Title: TRANSCRIPTIONAL ACTIVATION SYSTEM, ACTIVATORS, AND USES THEREFOR

Assistant Commissioner For Patents  
Washington, DC 20231

Sir:

**PRELIMINARY AMENDMENT**

The above-referenced Continuation patent application was filed in order to continue prosecution of a Parent application (USSN 08/850,127). A Final Office Action was mailed in that Parent application on October 27, 2000, and a Response to Final Office Action, which Response included an Amendment, was submitted on January 31, 2001. An Advisory Action mailed February 21, 2001 indicated that the Amendment included in the Response to Final Office Action was not entered into the application. The present Preliminary Amendment is intended to introduce that Amendment into the case, and also to ensure that the Application conforms with the requirements of 37 CFR §§ 1.821-1.825, relating to Sequence Listings.

**Amendment**

In the Specification:

Please amend the specification as follows:

On page 1, please delete the paragraph at lines 4-7 and insert the following paragraph:

### Priority Information

This application is a Continuation of co-pending U.S. Serial No. 08/850,127, filed May 1, 1997 and claiming benefit of U.S. Serial No. 60/017,016, filed May 3, 1996. Each of these prior applications is incorporated herein by reference in its entirety.

On page 4, please delete the paragraph at lines 3-4, and replace it with the following:

Figure 1 demonstrates the dependence between transcriptional activation and the order of amino acids in the inventive peptide activator, SEQ ID NO: 167. Peptides having the same composition as SEQ ID NO: 167, but different sequence orders, such as SEQ ID NO: 226 and SEQ ID NO: 227, produce substantially lower  $\beta$ -gal activity levels. As indicated, SEQ ID NO: 167 produces a  $\beta$ -gal activity level of 4400, while SEQ ID NO: 226 and SEQ ID NO: 227 produce  $\beta$ -gal activities of 100 and 17 respectively.

On page 4, please delete the paragraph at lines 5-6, and replace it with the following:

Figure 2 presents the results of  $\beta$ -galactosidase assays demonstrating how the inventive peptide activator, SEQ ID NO: 167, effects activity levels of mutagenized Gal-4 DNA binding domain residues. The unmutagenized Gal4 DNA binding domain is represented by SEQ ID NO: 228; mutagenized domains are listed consecutively from SEQ ID NO: 229 through SEQ ID NO: 237.

On page 4, please delete the paragraph at lines 7-8, and replace it with the following:

Figure 3 shows transcriptional activation by SEQ ID NO: 238, comprising Gal 4 residues 96-100 and SEQ ID NO: 167, when linked to the pho4 binding domain.

### In the Claims:

Please cancel claims 1 and 11-17.

Please amend claim 2 to read:

2. The transcriptional activator of claim 4, wherein the peptide is approximately 8-17 amino acids in length.

Please amend claim 3 to read:

3. The transcriptional activator of claim 4, wherein the peptide is 6, 8, 11, or 13 amino acids in length.

Please amend claim 4 to read:

4. A transcriptional activator comprising:

a DNA binding moiety; and

a transcription activation peptide that is at least approximately 25% hydrophobic and is between about 6 and 25 amino acids in length, which peptide is linked to the DNA binding moiety in a manner that does not interfere with its DNA binding activity, the transcription activation peptide being both necessary and sufficient to activate transcription, the transcriptional activator being characterized by an ability, when expressed in yeast cells, to activate transcription from a promoter including a recognition site for the DNA binding moiety approximately 250-1000 basepairs upstream of the transcription start site, the transcriptional activator being characterized by an inability to squelch transcriptional activation by LexA-Gal4 when expressed in yeast.

Please amend claim 6 to read:

6. The transcriptional activator of claim 4 or claim 5, which transcriptional activator, when expressed in yeast, does not squelch transcriptional activation by LexA-Gal11.

Please amend claim 8 to read:

8. The transcriptional activator of claim 4, wherein the peptide includes at least one aromatic amino acid.

Please amend claim 9 to read:

9. The transcriptional activator of claim 4, wherein the peptide does not include any basic amino acids.

Please amend claim 10 to read:

10. The transcriptional activator of claim 4, wherein the peptide is selected from the group consisting of LS4 (QLPPWL; SEQ ID NO: 8); LS8 (QFLDAL; SEQ ID NO: 16); LS11 (LDSFYV; SEQ ID NO: 21); LS12 (PPPPWP; SEQ ID NO: 23); LS17 (SWFDVVE; SEQ ID NO: 33); LS19 (QLPDLF; SEQ ID NO: 37); LS20 (PLPDLF; SEQ ID NO: 39); LS21 (FESDDI; SEQ ID NO: 41); LS24 (QYDLFP; SEQ ID NO: 45); LS25 (LPDLIL; SEQ ID NO: 47); LS30 (LPDFDP; SEQ ID NO: 55); LS35 (LFPYSL; SEQ ID NO: 57); LS51 (FDPFNQ; SEQ ID NO: 71); LS64 (DFDVLL; SEQ ID NO: 85); LS102 (HPPPPI; SEQ ID NO: 92); LS105 (LPGCFF; SEQ ID NO: 95); LS106 (QYDLFD; SEQ ID NO: 97); LS120 (YPPPPF; SEQ ID NO: 115); LS123 (PLPPFL; SEQ ID NO: 118); LS135 (LPPPWL; SEQ ID NO: 136); LS136 (VWPPAV; SEQ ID NO: 138); LS152 (DPPWYL; SEQ ID NO: 154); LS153 (LY; SEQ ID NO: 156); LS158 (FDPFGL; SEQ ID NO: 160); LS160 (PPSVNL; SEQ ID NO: 162); LS201 (YLLPTCIP; SEQ ID NO: 167); LS202 (LQVHNST; SEQ ID NO: 169); LS203 (VLDFTPFL; SEQ ID NO: 171); LS206 (HHAFYEIP; SEQ ID NO: 175); LS212 (PWYPTPYL; SEQ ID NO: 183); LS223

(YLLPFLPY; SEQ ID NO: 195); LS225 (YFLPLLST; SEQ ID NO: 199); LS232 (FSPTFWAF; SEQ ID NO: 209); LS241 (LIMNWPTY; SEQ ID NO: 221), each of these peptides extended at its amino terminal end by Gal4 residues 96-100, and each of these peptides extended at its amino terminal end by Gal4 96-100 except that one or both of Gal4 residues 99 and 100 has been substituted with a different amino acid.

### Remarks

As noted above, this Preliminary Amendment is being filed to introduce into the present case amendments that were submitted in the Parent Application but were not entered, and also to bring the present application into conformity with the Rules relating to Sequence Listings. Applicant respectfully submits that the presently pending claims are now in condition for allowance. The present claims recite transcriptional activators comprising a transcription activation *peptide*, i.e., a peptide that is both necessary and sufficient for transcriptional activation. Nowhere is such a transcriptional activator described in the cited references. In particular, the Himmelfarb et al. reference cited in the parent application describes a transcriptional activator comprising more than 900 amino acids of Gal11 fused to a DNA binding domain. There is no peptide portion of Gal11 that acts as a transcriptional activation peptide (i.e., that is necessary and sufficient for transcriptional activation). The smallest fragment of Gal11 that is known to activate transcription is 282 amino acids long (see Barberis et al. Cell 81:359, 1995, page 365, line 4). Thus, Gal11 simply does not include a transcription activation peptide that is “both necessary and sufficient to activate transcription” and “that is at least approximately 25% hydrophobic and is between about 6 and 25 amino acids in length,” as recited in claim 4.

In light of the distinctions presented above, the Applicants submit that the Himmelfarb et al. reference does not contain every element of the claimed invention, and thus is not anticipatory under 35 U.S.C. § 102(b). Applicants respectfully request withdrawal of this rejection.

Please charge any fees or credit any overpayments to our Deposit Account No. 03-1721.

Respectfully submitted,

Dated: October 3, 2001

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**Version with Markings to Show Changes Made**

2. (Amended) The transcriptional activator of claim [1] 4, wherein the peptide is approximately 8-17 amino acids in length.

3. (Amended) The transcriptional activator of claim [2] 4, wherein the peptide is 6, 8, 11, or 13 amino acids in length.

4. (Amended) A transcriptional activator comprising:  
a DNA binding moiety; and  
a transcription activation peptide that is at least approximately 25% hydrophobic and is between about 6 and 25 amino acids in length, which peptide is linked to the DNA binding moiety in a manner that does not interfere with its DNA binding activity, the transcription activation peptide being both necessary and sufficient to activate transcription,  
the transcriptional activator being characterized by an ability, when expressed in yeast cells, to activate transcription from a promoter including a recognition site for the DNA binding moiety approximately 250-1000 basepairs upstream of the transcription start site,  
the transcriptional activator being characterized by an inability to squelch transcriptional activation by LexA-Gal4 when expressed in yeast.

5. The transcriptional activator of claim 4, which transcriptional activator, when expressed in yeast, does not squelch transcriptional activation by LexA-Gal4.

6. (Amended) The transcriptional activator of claim 4 or claim 5, which transcriptional activator, when expressed in yeast, does not squelch transcriptional activation by LexA-Gal11.

7. The transcriptional activator of claim 4 or claim 5, in which the DNA binding moiety comprises Gal4(1-100) and the activator, when expressed in yeast, activates transcription at least half as well as does Gal4 from a promoter containing at least one Gal4 DNA binding site approximately 250-1000 basepairs upstream of the transcription start site.

8. (Amended) The transcriptional activator of [claim 1] or claim 4, wherein the peptide includes at least one aromatic amino acid.

9. (Amended) The transcriptional activator of [claim 1 or] claim 4, wherein the peptide does not include any basic amino acids.

10. (Amended) The transcriptional activator of [claim 1 and] claim 4, wherein the peptide is selected from the group consisting of LS4 (QLPPWL; SEQ ID NO: 8); LS8 (QFLDAL; SEQ ID NO: 16); LS11 (LDSFYV; SEQ ID NO: 21); LS12 (PPPPWP; SEQ ID NO: 23); LS17 (SWFDVE; SEQ ID NO: 33); LS19 (QLPDLF; SEQ ID NO: 37); LS20 (PLPDLF; SEQ ID NO: 39); LS21 (FESDDI; SEQ ID NO: 41); LS24 (QYDLFP; SEQ ID NO: 45); LS25 (LPDLIL; SEQ ID NO: 47); LS30 (LPDFDP; SEQ ID NO: 55); LS35 (LFPYSL; SEQ ID NO: 57); LS51 (FDPFNQ; SEQ ID NO: 71); LS64 (DFDVLL; SEQ ID NO: 85); LS102 (HPPPPI; SEQ ID NO: 92); LS105 (LPGCFF; SEQ ID NO: 95); LS106 (QYDLFD; SEQ ID NO: 97); LS120 (YPPPPF; SEQ ID NO: 115); LS123 (PLPPFL; SEQ ID NO: 118); LS135 (LPPPWL; SEQ ID NO: 136);

LS136 (VWPPAV; SEQ ID NO: 138); LS152 (DPPWYL; SEQ ID NO: 154); LS153 (LY; SEQ ID NO: 156); LS158 (FDPFGL; SEQ ID NO: 160); LS160 (PPSVNL; SEQ ID NO: 162); LS201 (YLLPTCIP; SEQ ID NO: 167); LS202 (LQVHNST; SEQ ID NO: 169); LS203 (VLDFTPFL; SEQ ID NO: 171); LS206 (HHAFYEIP; SEQ ID NO: 175); LS212 (PWYPTPYL; SEQ ID NO: 183); LS223 (YLLPFLPY; SEQ ID NO: 195); LS225 (YFLPLLST; SEQ ID NO: 199); LS232 (FSPTFWAF; SEQ ID NO: 209); LS241 (LIMNWPTY; SEQ ID NO: 221), each of these peptides extended at its amino terminal end by Gal4 residues 96-100, and each of these peptides extended at its amino terminal end by Gal4 96-100 except that one or both of Gal4 residues 99 and 100 has been substituted with a different amino acid.

**Claims Pending After Entrance of Present Amendment**

2. The transcriptional activator of claim 4, wherein the peptide is approximately 8-17 amino acids in length.
3. The transcriptional activator of claim 4, wherein the peptide is 6, 8, 11, or 13 amino acids in length.
4. A transcriptional activator comprising:
  - a DNA binding moiety; and
  - a transcription activation peptide that is at least approximately 25% hydrophobic and is between about 6 and 25 amino acids in length, which peptide is linked to the DNA binding moiety in a manner that does not interfere with its DNA binding activity, the transcription activation peptide being both necessary and sufficient to activate transcription, the transcriptional activator being characterized by an ability, when expressed in yeast cells, to activate transcription from a promoter including a recognition site for the DNA binding moiety approximately 250-1000 basepairs upstream of the transcription start site, the transcriptional activator being characterized by an inability to squelch transcriptional activation by LexA-Gal4 when expressed in yeast.
5. The transcriptional activator of claim 4, which transcriptional activator, when expressed in yeast, does not squelch transcriptional activation by LexA-Gal4.

6. The transcriptional activator of claim 4 or claim 5, which transcriptional activator, when expressed in yeast, does not squelch transcriptional activation by LexA-Gal11.

7. The transcriptional activator of claim 4 or claim 5, in which the DNA binding moiety comprises Gal4(1-100) and the activator, when expressed in yeast, activates transcription at least half as well as does Gal4 from a promoter containing at least one Gal4 DNA binding site approximately 250-1000 basepairs upstream of the transcription start site.

8. The transcriptional activator of or claim 4, wherein the peptide includes at least one aromatic amino acid.

9. The transcriptional activator of claim 4, wherein the peptide does not include any basic amino acids.

10. The transcriptional activator of claim 4, wherein the peptide is selected from the group consisting of LS4 (QLPPWL; SEQ ID NO: 8); LS8 (QFLDAL; SEQ ID NO: 16); LS11 (LDSFYV; SEQ ID NO: 21); LS12 (PPPPWP; SEQ ID NO: 23); LS17 (SWFDVE; SEQ ID NO: 33); LS19 (QLPDLF; SEQ ID NO: 37); LS20 (PLPDLF; SEQ ID NO: 39); LS21 (FESDDI; SEQ ID NO: 41); LS24 (QYDLFP; SEQ ID NO: 45); LS25 (LPDLIL; SEQ ID NO: 47); LS30 (LPDFDP; SEQ ID NO: 55); LS35 (LFPYSL; SEQ ID NO: 57); LS51 (FDPFNQ; SEQ ID NO: 71); LS64 (DFDVLL; SEQ ID NO: 85); LS102 (HPPPPI; SEQ ID NO: 92); LS105 (LPGCFF; SEQ ID NO: 95); LS106 (QYDLFD; SEQ ID NO: 97); LS120 (YPPPPF; SEQ ID NO: 115); LS123 (PLPPFL; SEQ ID NO: 118); LS135 (LPPPWL; SEQ ID NO: 136); LS136 (VWPPAV; SEQ ID NO: 137).

SEQ ID NO: 138); LS152 (DPPWYL; SEQ ID NO: 154); LS153 (LY; SEQ ID NO: 156); LS158 (FDPFGL; SEQ ID NO: 160); LS160 (PPSVNL; SEQ ID NO: 162); LS201 (YLLPTCIP; SEQ ID NO: 167); LS202 (LQVHNST; SEQ ID NO: 169); LS203 (VLDFTPFL; SEQ ID NO: 171); LS206 (HHAFYEIP; SEQ ID NO: 175); LS212 (PWYPTPYL; SEQ ID NO: 183); LS223 (YLLPFLPY; SEQ ID NO: 195); LS225 (YFLPLLST; SEQ ID NO: 199); LS232 (FSPTFWAF; SEQ ID NO: 209); LS241 (LIMNWPTY; SEQ ID NO: 221), each of these peptides extended at its amino terminal end by Gal4 residues 96-100, and each of these peptides extended at its amino terminal end by Gal4 96-100 except that one or both of Gal4 residues 99 and 100 has been substituted with a different amino acid.

Date Filed: October 3, 2061

The Patent and Trademark Office stamping  
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of documents identified as follows: via Certificate of Mailing

Applicant: Ptashne et al.

Serial No.: NOT YET ASSIGNED

Filing Date: August 31, 2001

Title:

## USES THEREFOR

- 1.) Transmittal Letter (1 sheet)
- 2.) 12 sheets of Preliminary Amendment
- 3.) Return Postcard

Attorney: BHJ      Attorney Docket No. 0342941-0063

